78

Tularemia Vaccines

Alan S. Cross and Eileen M. Barry
Center for Vaccine Development, University of Maryland School of Medicine, Baltimore, Maryland, U.S.A.

HISTORY/EPIDEMIOLOGY/CLINICAL

Tularemia, a zoonotic infection caused by *Francisella tularensis*, is an infection of the northern hemisphere with highly localized endemic foci. It was first reported by McCoy in 1911 as a disease of ground squirrels dying of a plague-like disease in Tulare County, California. The first reported human cases of tularemia were reported in 1914. Clinical disease most commonly manifests as a cutaneous infection in individuals exposed to infected animals or bitten by arthropods (e.g., ticks) that carry the bacteria. In 1942, Francis estimated that two-thirds of all American cases of tularemia were linked to contacts with cottontail rabbits (1). It has been stated that ''. . .no other infection of animals communicable to man . . . can be acquired from sources so numerous and so diverse'' (2).

While other routes of infection are more common, inhalational tularemia results in significant morbidity and occasional mortality. Before antibiotics, systemic infection with type A strains acquired following inhalation had a mortality rate of 30% to 60% (3). Although well-described sporadic cases have been acquired from the environment (e.g., gardeners at Martha's vineyard), widespread pulmonary tularemia is most likely thought to be acquired following its intentional release during a biological attack. In 1969, the World Health Organization estimated that aerosolization of 50 kg of *F. tularensis* in area of 5 million people would incapacitate 250,000 and kill 19,000 individuals (4). Using these data, the U.S. Centers for Disease Control and Prevention (CDC) concluded that such an attack would cost $5.4 billion/100,000 individuals affected (5). Such a high cost and morbidity led the CDC to designate *F. tularensis* as one of six category A select agents.

Bacteriology

Francis originally named the organism *Bacterium tularensis* in 1919; it was later designated *Pasteurella tularensis,* and finally renamed *Francisella tularensis* in 1947 (6). Subsequent sequence analysis of 16S rDNA resulted in its placement in *Proteobacteria*. With its high cell wall lipid content and unique cellular fatty acid composition, *Francisella* is the only recognized genus in the family *Francisellaceae*. There are four subspecies. *F. tularensis* subsp. (ssp.) *tularensis* (type A), the most virulent subspecies, is found only in North America. As few as 10 bacteria of *F. tularensis* ssp. *tularensis* can cause subcutaneous infection in man, while 25 organisms can do so by the aerosol route (7). A less virulent strain, *F. tularensis* ssp. *holarctica* (type B), is found in North America, Europe, and Asia, while strains from Central Asia have been designated *F. tularensis* ssp. *mediasiatica.* A fourth subspecies, *F. tularensis subsp novicida* is not considered a human pathogen.

Virulence

The manifestations of the disease are most likely associated with the host cellular inflammatory response induced by *F. tularensis* infection (8). Not only can *F. tularensis* infect phagocytes (macrophages, neutrophils, and dendritic cells), but also nonprofessional phagocytes such as hepatocytes, endothelial cells, and alveolar type II cells. Since *F. tularensis* is considered an intracellular organism, most of the work on its virulence has been conducted in macrophage cell cultures. *F. tularensis* does not produce any obvious exotoxins, and its lipopolysaccharide (LPS) is not endotoxic. One *Francisella* pathogenicity island (FPI) has been identified, which contains genes *iglA-D*, *pdpA*, and *pdpD* that encode proteins whose expression is regulated by macrophage growth locus genes, *mglA* and *mglB*. Within the macrophage, *F. tularensis* blocks phagosome maturation and acidification, and disrupts the phagosomal membrane which permits escape of *F. tularensis* into the cytoplasm where intracellular growth leads to both activation of caspase pathways and apoptosis (9). To exert its virulence both in vitro and in vivo, *F. tularensis* must escape from the phagosome, an event that depends on the expression of a pathogen-specific 23-kDa protein, encoded by intracellular growth locus gene, *iglC,* also located on the pathogenicity island. While its function is unknown, *iglC* loss results in complete loss of virulence as well as an inability to induce a protective immune response, perhaps through an inability to allow secreted proteins to enter the histocompatibility complex (MHC) class I pathway. It has been observed that *iglC* mutants of *F. tularensis* remain within the phagosome, where they are still able to induce a robust TLR2 response, but are unable to induce apoptosis (10).

Genes that do not appear to influence the behavior of *F. tularensis* in macrophages, such as a putative type IV pilin gene or *tolC,* may also play a role in virulence (11,12). Type IV pili are virulence factors for many bacteria. Genes encoding the type IV pili were identified in the ssp. *tularensis* strain, Schu S4 genome sequence, but its precise role in infection is not known. Type IV pili appear to be involved in the dissemination of ssp. *holarctica* from its initial site of infection.

The O-polysaccharide (PS) of the LPS, which is common to ssp. *tularensis* and *holarctica*, is essential for virulence. The